Dual feedback loops in the *GAL* regulon suppress cellular heterogeneity in yeast
Supplementary Note

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Model Description

In this section we briefly present our mathematical model of the yeast galactose utilization pathway. This model is based on an earlier published model [1], with some simplifications. The simulation results in this study were obtained using the chemical kinetics modeling program Dizzy [2]. This software is available (under a free and open-source license) on the World Wide Web at `http://magnet.systemsbiology.net/software/Dizzy`.

The species names in the model are defined in Table 1. In addition to the dynamical symbols defined in Table 1, the symbol G_{ex} (shown as GAE in the text) is used to denote the concentration of extracellular galactose. G_{ex} is a free parameter in the model. A species symbol appearing in the kinetic equations of the model represents a cell population average number of molecules per unit volume, where the unit of volume is 3.57×10^{-14} liters (a typical cell volume for haploid yeast [1]). Similarly, "molec" appearing as a unit in the kinetic constants for the model represents molecules per unit volume as defined above. [The exception to this is the case of Gal80p, for which the number of molecules in the cytoplasm and in the nucleus are

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Symbol	*Symbol in* [1]	*Explanation*	*Initial data (molec.)*
R_1	R1	*GAL1* mRNA	0.2647
R_2	R2	*GAL2* mRNA	0.3305
R_3	R3	*GAL3* mRNA	0.9044
R_4	R4	*GAL4* mRNA	0.4
R_{80}	R80	*GAL80* mRNA	1.1871
R_{rep}	n/a	*reporter* mRNA	0.2647
G_1	G1	Gal1p	132.3267
G_2	G2	Gal2p	1156.7
G_3	G3	Gal3p	4341.2
G_{3i}	G3i	Gal3p (activated by galactose)	0
G_4	G4	Gal4p (monomer)	0.1563
G_{4d}	G4d	Gal4p (homodimer)	308.92
G_{80}	G80	Gal80p (monomer), nucleus	0.1138
G_{80C}	n/a	Gal80p (monomer), cytoplasm	0.1095
G_{80d}	G80d	Gal80p (homodimer), nucleus	157.229
G_{80Cd}	n/a	Gal80p (homodimer), cytoplasm	157.229
G_{rep}	n/a	GFP reporter	132.327
$C_{3i,80}$	G80G3i	complex of Gal3p(activated) and Gal80Cd	0
G_{ic}	GAI	intracellular galactose	0

Table 1: Table of species names in the model of the galactose utilization pathway. The "Symbol in [1]" column indicates how the species are referred to in the early version of the model [1]. The "Symbol" column refers to the mathematical symbol for the species as it appears in this Note. The column "Initial data" is the steady-state fixed point of this system of equations (for the wild-type strain), with zero external galactose (i.e., uninduced system) at steady-state. Here, the "molec" denotes molecules per unit volume, in units of the average haploid cell volume (see above).

represented by distinct symbols.] We used the conversion factor of 4.65×10^{-8} millimolar/(molec/cell).

In the model, the rate constants for degradation of mRNA were taken from [3, 4]. The rate constants for degradation of proteins were estimated from [5–8], with a minimum rate constant given by the dilution rate of 0.00385 min^{-1} based on an estimated rate of cell budding of once per 180 minutes on galactose (data not shown). The maximum rates of initiation of transcription were calculated from the maximum mRNA levels [1, 9] and the known degradation rate constants for mRNA. The maximum concentration of *GAL3* mRNA was adjusted so that the total amount of Gal3p is approximately 5 times the total amount of Gal80p [10]. The rate constants of initiation of translation were obtained from the known rate constants of degradation for proteins, and the protein-to-mRNA ratios published in [6, 11]. The equilibrium dissociation constant for galactose activation of Gal3p was taken from [12]. The kinetic parameters for galactose transport and galactokinase activity were taken as published in [1, 13]. The kinetic parameters for dimerization were adjusted to ensure that Gal80p dimerizes with high affinity and that the dimer is stabilized when bound on Gal4p [12, 14]. The equilibrium dissociation constant for Gal3p*-Gal80p complex formation was the remaining important free parameter in the model; it was adjusted to give a fractional activity for the reporter gene consistent with the results of [15].

Because the bi-functional protein Gal1p is 40 times less effective than its homolog Gal3p at activating the *GAL* switch in response to galactose [16], we did not include its weak potential feedback role in the model. Because we are not considering growth on glucose, our model does not include glucose repression through *MIG1*. The growth rates for mutant and wild-type strains were assumed to be the same, which is consistent with the results of a comparative growth experiment carried out in 2% galactose and 2% raffinose (results not shown). We assume cooperativity to explain the proposed stabilization of Gal80p binding at adjacent Gal4p binding sites [14]. The half-life of the reporter (GFP) was taken to be 60 minutes, based on the observed rate of falloff of GFP expression in the wild-type time-course flow cytometry data for 0.1% initial galactose. The average time scale for budding was taken to be 180 minutes in galactose (data not shown), which was used to calculate the volume dilution rate constant in the model. The estimates of the fractional activity of the *CYC1*-driven genes in the mutant strain, were based on [17, 18]. Our model takes into account galactose uptake only through the high-affinity, inducible galactose transporter Gal2p, and not the low-affinity or constitutive galactose transport processes [19, 20].

The results shown in **Supplementary Figure 2** were obtained using a quasi-analytic solution to the kinetic model as a function of the intracellular galactose concentration, assuming that all protein-protein interactions are at quasi-steady-state (QSS). The kinetic model was reduced symbolically using Mathematica, and the resulting nonlinear equations for the galactose import rate were solved numerically.

The dynamical equations of the model are as follows:

$$\frac{dR_1}{dt} = k_{ir,\mathrm{gal1}} F_4(K_P G_{4d}, K_Q G_{80d}, q_r) - k_{dr,\mathrm{gal1}} R_1 \quad (1)$$

$$\frac{dR_2}{dt} = k_{ir,\mathrm{gal2}} F_5(K_P G_{4d}, K_Q G_{80d}, q_r) - k_{dr,\mathrm{gal2}} R_2 \quad (2)$$

$$\frac{dR_3}{dt} = k_{ir,\mathrm{gal3}} q_3 F_1(K_P G_{4d}, K_Q G_{80d}) - k_{dr,\mathrm{gal3}} R_3 \quad (3)$$

$$\frac{dR_{rep}}{dt} = k_{ir,\mathrm{rep}} F_4(K_P G_{4d}, K_Q G_{80d}, q_r) - k_{dr,\mathrm{rep}} R_{rep} \quad (4)$$

$$\frac{dR_{80}}{dt} = k_{ir,\mathrm{gal80}} F_1(K_P G_{4d}, K_Q G_{80d}) - k_{dr,\mathrm{gal80}} R_{80} \quad (5)$$

$$\frac{dG_1}{dt} = k_{ip,\mathrm{gal1}} R_1 - k_{dp,\mathrm{gal1}} G_1 \quad (6)$$

$$\frac{dG_2}{dt} = k_{ip,\mathrm{gal2}} R_2 - k_{dp,\mathrm{gal2}} G_2 \quad (7)$$

$$\frac{dG_3}{dt} = k_{ip,\mathrm{gal3}} R_3 - k_{dp,\mathrm{gal3}} G_3 - k_{fi} G_3 G_{ic} + k_{ri} G_{3i} \quad (8)$$

$$\frac{dG_{3i}}{dt} = k_{fi} G_3 G_{ic} - k_{ri} G_{3i} - k_{dp,\mathrm{gal3}} G_{3i} - k_{fd,3i,80} G_{80Cd} G_{3i} + k_{rd,3i,80} C_{3i,80} \quad (9)$$

$$\frac{dG_4}{dt} = k_{ip,\mathrm{gal4}} R_4 - k_{dp,\mathrm{gal4}} G_4 - 2k_{fd} G_4^2 + 2k_{rd} G_{4d} \quad (10)$$

$$\frac{dG_{4d}}{dt} = k_{fd} G_4^2 - k_{rd} G_{4d} - k_{dp,\mathrm{gal4}} G_{4d} \quad (11)$$

$$\frac{dG_{rep}}{dt} = k_{ip,\mathrm{rep}} R_{rep} - k_{dp,\mathrm{rep}} G_{rep} \quad (12)$$

$$\frac{dG_{80}}{dt} = k_{ip,\mathrm{gal80}} R_{80} - k_{dp,\mathrm{gal80}} G_{80} - k_{f80} G_{80} + k_{r80} G_{80C} - 2k_{fd} G_{80}^2 + 2k_{rd} G_{80d} \quad (13)$$

$$\frac{dG_{80C}}{dt} = k_{f80} G_{80} - k_{r80} G_{80C} - 2k_{fd} G_{80C}^2 + 2k_{rd} G_{80Cd} - k_{dp,\mathrm{gal80}} G_{80C} \quad (14)$$

$$\frac{dG_{80d}}{dt} = k_{fd} G_{80}^2 - k_{rd} G_{80d} - k_{dp,\mathrm{gal80}} G_{80d} - k_{f80} G_{80d} \quad (15)$$

$$
\begin{aligned}
& & & +k_{r80}G_{80Cd} \\
\frac{dG_{80Cd}}{dt} &= & & k_{fd}G_{80C}^2 - k_{rd}G_{80Cd} - k_{dp,\mathrm{gal80}}G_{80Cd} + k_{f80}G_{80d} \qquad (16) \\
& & & -k_{r80}G_{80Cd} - k_{fd,3i,80}G_{80Cd}G_{3i} + k_{rd,3i,80}C_{3i,80} \\
\frac{dC_{3i,80}}{dt} &= & & k_{fd,3i,80}G_{80Cd}G_{3i} - k_{rd,3i,80}C_{3i,80} - 0.5k_{dp,\mathrm{gal3}}C_{3i,80} \qquad (17) \\
\frac{dG_{ic}}{dt} &= & & v_{\mathrm{TR}} - v_{\mathrm{GK}} - k_{fi}G_3G_{ic} + k_{ri}G_{3i} \qquad (18)
\end{aligned}
$$

Time t is in units of minutes. In the first five equations above, the following fractional activation functions are used:

$$
\begin{aligned}
F_1(P,Q) &= \frac{P}{1+P+PQ} \qquad (19) \\
F_4(P,Q,q) &= \frac{F_4(P,Q,q)_{\mathrm{num}}}{F_4(P,Q,q)_{\mathrm{denom}}} \qquad (20) \\
F_4(P,Q,q)_{\mathrm{num}} &= 4P + 12P^2Q + 6P^2 + 4P^3 + 12P^3Q + 12qP^3Q^2 \\
& \quad +P^4 + 4P^4Q + 4q^2P^4Q^3 + 6qP^4Q^2 \qquad (21) \\
F_4(P,Q,q)_{\mathrm{denom}} &= F_4(P,q,q)_{\mathrm{num}} + 1 + 4PQ + 6qP^2Q^2 + 4q^2P^3Q^3 \\
& \quad +q^3P^4Q^4 \qquad (22) \\
F_5(P,Q,q) &= \frac{F_5(P,Q,q)_{\mathrm{num}}}{F_5(P,Q,q)_{\mathrm{denom}}} \qquad (23) \\
F_5(P,Q,q)_{\mathrm{num}} &= 5P + 10P^2 + 20P^2Q + 30qP^3Q^2 + 30P^3Q \\
& \quad +10P^3 + 5P^4 + 30qP^4Q^2 + 20P^4Q + 20q^2P^4Q^3 \\
& \quad +P^5 + 5P^5Q + 5q^3P^5Q^4 + 10q^2P^5Q^3 \\
& \quad +10qP^5Q^2 \qquad (24) \\
F_5(P,Q,q)_{\mathrm{denom}} &= F_5(P,Q,q)_{\mathrm{num}} + 1 + 5PQ + 10qP^2Q^2 \\
& \quad +10q^2P^3Q^3 + 5q^3P^4Q^4 + q^4P^5Q^5 \qquad (25)
\end{aligned}
$$

The subscript on the F functions indicates the number of *Gal4p* binding sites in the promoter. The model for the mutant strain is identical to the above model, except that Equations (3) and (5) are replaced by:

$$
\begin{aligned}
\frac{dR_3}{dt} &= k_{ir,\mathrm{gal3}}f_{\mathrm{mut}} - k_{dr,\mathrm{gal3}}R_3 \qquad (26) \\
\frac{dR_{80}}{dt} &= k_{ir,\mathrm{gal80}}f_{\mathrm{mut}} - k_{dr,\mathrm{gal80}}R_{80} \qquad (27)
\end{aligned}
$$

where f_{mut} is a fixed dimensionless activation level, which we varied between 0.04–0.06 in our various simulations.

In Equations (21)–(24), the metabolic reaction velocities are defined as:

$$v_{\mathrm{TR}} = \frac{k_{\mathrm{TR}} G_2 (G_{ex} - G_{ic})}{(K_{m,\mathrm{TR}} + G_{ex} + G_{ic} + \frac{\alpha_{\mathrm{TR}}}{K_{m,\mathrm{TR}}} G_{ex} G_{ic})} \tag{28}$$

$$v_{\mathrm{GK}} = \frac{k_{cat,\mathrm{GK}} G_1 G_{ic}}{K_{m,\mathrm{GK}} + G_{ic}} \tag{29}$$

$$\tag{30}$$

The kinetic parameters of the model are as follows:

$$\begin{aligned}
k_{ir,\mathrm{gal1}} &= 0.7379 \ \text{molec min}^{-1} \\
k_{ir,\mathrm{gal2}} &= 2.542 \ \text{molec min}^{-1} \\
k_{ir,\mathrm{gal3}} &= 0.7465 \ \text{molec min}^{-1} \\
k_{ir,\mathrm{gal4}} &= 0.009902 \ \text{molec min}^{-1} \\
k_{ir,\mathrm{gal80}} &= 0.6065 \ \text{molec min}^{-1} \\
k_{ir,\mathrm{rep}} &= 1.1440 \ \text{molec min}^{-1} \\
q_3 &= 0.571, \ \ (\mathrm{wild-type}) \\
q_3 &= 1.0, \ \ (\mathrm{mutant}) \\
K_P &= 0.02104 \\
K_Q &= 0.1052 \\
q_r &= 30.0 \\
k_{ip,\mathrm{gal1}} &= 1.9254 \ \text{min}^{-1} \\
k_{ip,\mathrm{gal2}} &= 13.4779 \ \text{min}^{-1} \\
k_{ip,\mathrm{gal3}} &= 55.4518 \ \text{min}^{-1} \\
k_{ip,\mathrm{gal4}} &= 10.7091 \ \text{min}^{-1} \\
k_{ip,\mathrm{gal80}} &= 3.6737 \ \text{min}^{-1} \\
k_{ip,\mathrm{rep}} &= 5.7762 \ \text{min}^{-1} \\
k_{dr,\mathrm{gal1}} &= 0.02236 \ \text{min}^{-1} \\
k_{dr,\mathrm{gal2}} &= 0.07702 \ \text{min}^{-1} \\
k_{dr,\mathrm{gal3}} &= 0.02666 \ \text{min}^{-1} \\
k_{dr,\mathrm{gal4}} &= 0.02476 \ \text{min}^{-1} \\
k_{dr,\mathrm{gal80}} &= 0.02888 \ \text{min}^{-1} \\
k_{dr,\mathrm{rep}} &= 0.03466 \ \text{min}^{-1} \\
k_{dp,\mathrm{gal1}} &= 0.003851 \ \text{min}^{-1} \\
k_{dp,\mathrm{gal2}} &= 0.003851 \ \text{min}^{-1}
\end{aligned}$$

$$
\begin{aligned}
k_{dp,\mathrm{gal3}} &= 0.01155\ \mathrm{min}^{-1} \\
k_{dp,\mathrm{gal4}} &= 0.006931\ \mathrm{min}^{-1} \\
k_{dp,\mathrm{gal80}} &= 0.006931\ \mathrm{min}^{-1} \\
k_{dp,\mathrm{rep}} &= 0.01155\ \mathrm{min}^{-1} \\
k_{fd} &= 0.001\ \mathrm{molec}^{-1}\mathrm{min}^{-1} \\
k_{rd} &= 100\ \mathrm{min}^{-1} \\
k_{fi} &= 7.45 \times 10^{-7}\ \mathrm{molec}^{-1}\mathrm{min}^{-1} \\
k_{ri} &= 890.0\ \mathrm{min}^{-1} \\
k_{f80} &= 500\ \mathrm{min}^{-1} \\
k_{r80} &= 500\ \mathrm{min}^{-1} \\
k_{fd,\mathrm{3i,80}} &= 0.01596\ \mathrm{molec}^{-1}\mathrm{min}^{-1} \\
k_{rd,\mathrm{3i,80}} &= 0.02572\ \mathrm{min}^{-1} \\
\alpha_{\mathrm{TR}} &= 1 \\
K_{m,\mathrm{TR}} &= 2.1505 \times 10^{7}\ \mathrm{molec} \\
k_{\mathrm{TR}} &= 4350\ \mathrm{min}^{-1} \\
k_{cat,\mathrm{GK}} &= 3350\ \mathrm{min}^{-1} \\
K_{m,\mathrm{GK}} &= 1.2903 \times 10^{7}\ \mathrm{molec}
\end{aligned}
$$

Quantities for which units are not given, are dimensionless. The symbol R_4 in Eq. (10) denotes the concentration of the *GAL4* mRNA in molecules per cell volume unit (see Table 1).

The dimensionless parameter q_3 is adjusted between 0.571 for the wild-type and 1.0 for the mutant strain, to account for the observation that the mutant strain steady-state cell population-average induction level is approximately 1.3-fold higher than the wild-type strain (with identical *GAL3* and *GAL80* promoters in the mutant strain).

The steady-state fixed point of this system of equations (for the wild-type strain), with zero external galactose (i.e., $G_{ex} = 0$), is given by the values in Table 1. These values are used as the initial conditions to model cells pregrown in a non-inducing, non-repressing carbon source such as 2% raffinose.

Sigmoidal fit to time-course data

From the time-course fluorescence measurements (see **Fig. 2a** in the main text), we estimated the fraction of responding cells by computing the fraction of flow cytometry events in which the fluorescence is greater than three times the autofluorescence intensity of the control strain. The resulting measurements for mutant and wild-type are shown as data points in **Fig. 2b** in the main text. In order to estimate the time-scale for the increase in the fraction of responding cells, we fit a sigmoidal function of time to each data set (mutant and wild-type),

$$F(t) = \frac{At^n}{t^n + B^n}.$$

Here, n is a steepness coefficient, A is the asymptotic fraction of responding cells (assuming no media depletion), t is the elapsed time, and B controls the time at which the system is 50% induced. The fit parameters are shown in Table 2. The best-fit sigmoidal functions are plotted as curves in **Fig. 2b** in the main text.

strain	A	B (hours)	n
wild-type	0.995	1.595	6.64
mutant	0.906	2.518	2.898

Table 2: Parameters for the best-fit sigmoidal function to the time-course fraction of responding cells grown in 0.1% galactose. The fit parameters show that the time-scale for 50% of the cells to respond in the mutant strain, is 70% longer than in the wild-type.

Statistical analysis

We carried out a statistical test of the null hypothesis, namely, that the fluorescence coefficient of variation measurements obtained for the mutant and wild-type come from the same distribution. We carried out a two-tailed t-test using the Statistics Toolbox in MATLAB (Mathworks). Separate tests were performed for the 0.1% and 0.2% galactose cases. Four replicates were conducted per strain (and condition) and thus, each test had six degrees of freedom. The test was performed without assuming equal variances of the two data sets.

conc (g / 100 mL)	⟨WT CV⟩	⟨Mut CV⟩	$t_\star$	p-value
0.1	0.252 ± 0.007	0.334 ± 0.033	4.92	0.0027
0.2	0.245 ± 0.015	0.316 ± 0.030	4.34	0.0049

Table 3: Table summarizing the test of significance of the deviation between the mutant CV (coefficient of variation) and the wild-type CV, for fluorescence at 6 hours. We are testing the null hypothesis that the distributions of the wild-type and mutant reporter expression CV at 6 hours, are identical, i.e., that the mutant and wild-type strain have equal cell-to-cell heterogeneity of *GAL* response at 6 hours. Here, "conc" means the galactose concentration; "WT" means wild-type; and "Mut" means mutant. Angle brackets denote an average over four replicates. The symbol $t_\star$ means the number of standard deviations by which the mutant and wild-type average CVs differ (using the composite uncertainty). The column "p-value" indicates the confidence with which we can invalidate the null hypothesis, using the two-tailed t-test.

Forward Light Scatter

Using flow cytometry, we measured the forward light scatter of both wild-type and dual-loop-knockout strains (see **Methods** in the main text) to determine the approximate cell size of the strains during growth in raffinose and galactose. The results are shown in Table 4.

Simulations

The stochastic [21] and deterministic dynamics of the kinetic model was solved using the chemical kinetics software Dizzy[2]. Deterministic simulation were carried out using the Dormand-Prince ODE solver[22] with a fourth-order error estimation formula and adaptive step-size control. The ODE solver implementation used within Dizzy is the odeToJava package [23, 24]. The stochastic simulation solvers used within Dizzy were the Gillespie Tau-Leap [25] and the Gibson-Bruck [26] algorithms. The Gibson-Bruck solver was used for the raffinose case only. A stop time of 8000 minutes was used, with 400 time samples per simulation. The Tau-Leap relative error threshold was set to 0.002. For each condition, the number of separate simulations conducted was 12 (for mutant and wild-type models separately), yielding an effective ensemble size of 4800. Stochastic simula-

conc (g / 100 mL)	wild-type	mutant
0.00	1.08 ± 0.018	1.003 ± 0.985
0.05	0.998	0.996
0.1	1.031 ± 0.020	1.022 ± 0.032
0.2	1.030 ± 0.024	1.021 ± 0.031
0.5	0.992	0.991
1.0	0.997	0.985

Table 4: Average forward light scatter in the mutant and wild-type strains, relative to the control strain, after 6 hours of growth on galactose at the indicated concentration (except 0.0% galactose, when the measurement was performed immediately after transfer from 2% raffinose). Uncertanties are calculated over four replicates (for 0.1% and 0.2% galactose), and three replicates (for 0%).

tion results were cross-checked with steady-state noise estimates obtained using an eigenvalue decomposition of the Jacobian [27].

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